

I CLAIM:

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An injectable vaccine for treatment of Pythiosis which comprises in a sterile aqueous solution an admixture of:

(a) intracellular proteins separated from disrupted cells of *Pythium insidiosum*; and

(b) extracellular proteins from a supernatant from growing the cells of the *Pythium insidiosum*.

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The vaccine of Claim 1 wherein the proteins have been provided by (1) growing cells of the *Pythium insidiosum* in a culture medium, then killing the cells, then separating the killed cells from the culture medium so as to produce a first supernatant and then disrupting the cells in water to provide the intracellular proteins in a second supernatant which are separated and (2) separating the extracellular proteins from the first supernatant.

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The vaccine of Claim 2 wherein the cells have been disrupted by sonication.

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The vaccine of Claim 1 wherein the *Pythium insidiosum* is deposited as ATCC 58643.

-5-

The vaccine of Claims 2 or 3 wherein the culture medium is Sabouraud dextrose broth.

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The vaccine of Claim 2 wherein the cells are killed with thimersol.

-7-

The vaccine of Claim 2 wherein the disrupted cells are separated from the culture medium by centrifugation.

-8-

The vaccine of Claim 2 wherein the proteins have been separated by being precipitated together using acetone and then the precipitate is then dispersed in sterile distilled water, then dialyzed to remove low molecular weight components less than 10,000 MW to provide the vaccine.

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A method for providing an injectable vaccine for treatment of Pythiosis which comprises:

- (a) growing cells of *Pythium insidiosum* in a culture medium;
- (b) separating the cells from a first supernatant of the culture medium which contains extracellular proteins;
- (c) killing the cells;
- (d) disrupting the cells in sterile water;
- (e) separating the disrupted cells from the water to produce a second supernatant containing intracellular proteins;
- (f) mixing the first supernatant of step (b) with the second supernatant of step (e);
- (g) separating the combined proteins from the mixture of step (f);
- (h) mixing the separated proteins in sterile distilled water; and
- (i) dialyzing the mixture of step (h) to remove low molecular weight components less than 10,000 MW to produce the vaccine.

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The method of Claim 9 wherein the cells are disrupted by sonication.

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The method of Claim 9 wherein the *Pythium insidiosum* is deposited as ATCC 58643.

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The method of any one of Claims 9, 10 or 11 wherein the culture medium is Sabouraud dextrose broth.

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The method of Claim 9 wherein the cells are killed with thimersol.

-14-

The method of Claim 9 wherein the disrupted cells are separated from the water in step (e) by centrifugation.

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The method of Claim 9 wherein the separated proteins are separated in step (g) by being precipitated together using acetone from the first and second supernatants combined together.

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Sub B2 A method for treatment of Pythiosis in human patients having the disease which comprises:

(a) providing a vaccine containing separated proteins of *Pythium insidiosum* in a sterile aqueous solution; and

(b) vaccinating the patient with the vaccine.

Sub G2

The method of Claim 16 wherein the vaccination is subcutaneous.

Sub B3

A method for the treatment of Pythiosis in a mammal having the disease which comprises:

(a) providing an injectable vaccine which comprises in a sterile aqueous solution in admixture:

(1) an intracellular proteins separated from disrupted cells of *Pythium insidiosum*; and

(2) extracellular proteins from a supernatant from growing the cells of the *Pythium insidiosum*; and

(b) vaccinating the mammal with the vaccine.

Sub B4

The method of Claim 18 wherein in the proteins have been provided by growing cells of the *Pythium insidiosum* in a culture medium, then killing the cells, then separating the killed cells from the culture medium to produce a first supernatant and then disrupting the cells in water to provide the intracellular proteins in a second supernatant which have separated and (2) separating the extracellular proteins from the first supernatant.

The method of Claim 18 wherein the cells have been disrupted by sonication.

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The method of Claim 18 wherein the *Pythium insidiosum* is deposited as ATCC 58643.

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The method of any one of Claims 19, 20 or 21 wherein the culture medium is Sabouraud dextrose broth.

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The method of Claim 19 wherein the cells are killed with thimersol.

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The method of Claim 19 wherein the disrupted cells are separated from the culture medium for the cells by centrifugation.

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The method of Claim 19 wherein the separated proteins have been precipitated together from the first and second supernatants combined together using acetone and then dispensed in sterile distilled water to provide the vaccine.

Add B⁸